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Pharmacogenetics of nuclear receptors and drug transporters in inflammatory bowel disease and hepatic drug metabolism

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HABILITATIONSSCHRIFT

Pharmacogenetics of Nuclear Receptors and Drug Transporters in Inflammatory Bowel Disease and hepatic drug metabolism

zur Erreichung der *Venia Legendi*
der Universität Zürich

vorgelegt von
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Zürich, 30. Oktober 2011

Klinik für Klinische Pharmakologie und Toxikologie
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1. Publications

1.1 Five selected publications representing the "Kumulative Habilitationsschrift"

1. **Mwinyi J**, Johne A, Bauer S, Roots I and Gerloff T (2004) Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics. *Clin Pharmacol Ther* **75**:415-421.
IF (2010)=6.378
2. **Mwinyi J**, Cavaco I, Pedersen RS, Persson A, Burkhardt S, Mkrtchian S and Ingelman-Sundberg M (2010) Regulation of CYP2C19 expression by estrogen receptor alpha. Implications for estrogen dependent inhibition of drug metabolism. *Mol Pharmacol* **78**:886-94.
IF (2010)=4.725
3. Eloranta JJ*, Wenger C*, **Mwinyi J**, Hiller C, Gubler C, Vavricka SR, Fried M, Kullak-Ublick GA, and the Swiss IBD Cohort Study Group (2011) Association of a common vitamin D-binding protein (DBP) polymorphism with inflammatory bowel disease. *Pharmacogenet Genomics*. 2011 Sep;21(9):559-564.
IF (2010)=3.865
4. **Mwinyi J**, Wenger C, Eloranta JJ and Kullak-Ublick GA (2010) Glucocorticoid receptor gene haplotype structure and steroid therapy outcome in IBD patients. *World J Gastroenterol* **16**:3888-3896.
IF (2010)=2.092
5. **Mwinyi J***, Grete-Wenger C*, Eloranta JJ, and Kullak-Ublick GA (2011) The impact of PPAR γ genetic variants on IBD susceptibility and IBD disease course. Accepted for publication in the Journal *PPAR Research*
IF (2010)=2.798

1.2 Three publications of high scientific value, not discussed in the "Habilitationsschrift"

6. Baldwin RM*, Ohlsson S*, Pedersen RS*, **Mwinyi J**, Ingelman-Sundberg M, Eliasson E and Bertilsson L (2008) Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. *Br J Clin Pharmacol* **65**:767-774.
IF (2010)=3.063
7. **Mwinyi J***, Nekvindova J*, Cavaco I, Hofmann Y, Pedersen RS, Landman E, Mkrtchian S and Ingelman-Sundberg M (2010c) New insights into the regulation of CYP2C9 gene expression: the role of the transcription factor GATA-4. *Drug Metab Dispos* **38**:415-421.
IF (2010)=3.716
8. **Mwinyi J***, Cavaco I*, Yurdakok B, Mkrtchian S and Ingelman-Sundberg M (2011) The ligands of estrogen receptor α regulate cytochrome P450C9 (CYP2C9) expression. *J Pharmacol Exp Ther*. 2011 Jul;338(1):302-9.
IF (2010)=4.017

* denotes equal contribution of the authors; IF = impact factor

2. General background to the presented work and current state of research

2.1 Pharmacogenetics

Genetic variants are known to cause or contribute to human illness and to modulate patients' individual responses to medical treatments, as investigated in the research field of pharmacogenetics. Adverse drug events (ADEs) are a major safety risk in the use of medications and are estimated to cause 6-7% of all hospitalizations. They impose a considerable pharmacoeconomic burden on the health insurance system, prolonging hospital stay by an average of 2 days. Many genetic variants, especially genes encoding for cytochrome P450 enzymes or for hepatic transporters, such as in the OATP uptake transporter family have been shown to essentially influence the individual susceptibility for drug side effects [1]. Important examples include the genetic variants *CYP2C9**2 and *3, which determine slow metabolism towards coumarin derivatives and which are connected with a significant higher risk for bleeding events, or *CYP2D6* polymorphisms, which are able to modulate the relapse times of breast cancer under therapy with tamoxifen [2; 3]. These are typical examples for the often occurring scenario, where genetic variants directly modulate the efficacy of the drug metabolizing enzyme by inducing amino acid exchanges or by modulating the translation rate, introducing premature stop codons or splice defects into the genetic sequence. Beside this, the efficacy of drug metabolizing enzymes can be changed by genetic variants that occur within genes coding for transcription factors that are responsible for adequate gene regulation.

2.2 Polymorphically expressed drug transporters and CYP enzymes: OATP1B1 and CYP2C19

Liver organic anion transporting polypeptides (OATPs) are important proteins involved in the active uptake of drugs, xenobiotics, and amphipathic endogenous compounds from portal venous into the liver. They thereby initiate the hepatic first-pass metabolism and biliary elimination of a variety of compounds. The OATP family member OATP1B1 (coding gene: *SLCO21A6*) is exclusively expressed at the basolateral membrane of hepatocytes. OATP1B1 is known to be involved in the active uptake of a variety of different drugs into the liver, including 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors such as pravastatin, the antihistamine fexofenadine, and the angiotensin converting enzyme inhibitors enalapril and temocaprilat [4-6]. Various single nucleotide polymorphisms (SNPs) within *SLCO1B1* have been identified by different research groups [7; 8]. As shown by the habilitant [9] especially 3 non-synonymous SNPs, the genetic variants *SLCO1B1**1b, *3 and *5 occur in a high frequency within the *SLCO1B1* gene and are able to induce a modulatory effect on the OATP1B1 drug transport efficacy and drug safety with clinical consequences. It could be shown that carriers of the genetic variant *SLCO1B1**5 are at significantly higher risk to develop – partly severe - myopathies under statin therapy [10].

The Cytochrome P450 family member CYP2C19 is mainly expressed in the liver [11; 12] and is estimated to be involved in the metabolism of approximately 5% of drugs currently used in clinical practice [13; 14]. Drugs, which are mainly metabolized by CYP2C19 include proton pump inhibitors (e.g. omeprazole, pantoprazole), diazepam, selective serotonin reuptake inhibitors (SSRI) such as citalopram and escitalopram, and the anti-coagulant clopidogrel [1; 15-18]. The coding gene *CYP2C19* is highly polymorphically expressed leading to different drug metabolizer phenotypes. According to their enzyme activity individuals are divided into ultrafast metabolizers (homozygous carriers of the *CYP2C19*17* allelic variant) extensive metabolizers (EM; homozygous or heterozygous carriers of the wild type allele) and slow metabolizers (individuals homozygous for the variants *CYP2C19*2* or **3*) with clinical consequences for therapy safety and efficacy. While slow metabolizers have been shown to be at higher risk for thrombotic events due to a less effective transformation of the prodrug clopidogrel into the antithrombotic acting metabolite [19; 20], while ultrafast metabolizer phenotype appears to have a protective effect against thrombotic occurrences [21].

2.3 The transcription factor family of Nuclear Receptors

An important group of transcription factors displays the Nuclear Receptor family, which is involved in the regulation of a wide range of metabolic pathways, such as e.g. insulin sensitization of different tissues and lipid metabolism. Ligand-dependent activation enables them to rapidly induce transcription changes within the target cells. Currently, there are around 50 different nuclear receptors known. NRs have several characteristics in common. They are structurally composed of six functional regions (A–F), which are characterized by a various degree of sequence conservation. Most importantly, the highly conserved C region comprises the DNA-binding domain (DBD), which is composed of two zinc fingers, and the conserved E region, which contains the ligand-binding domain (LBD) [22]. Based on their ligands, NRs can be grouped into three subfamilies: The first subfamily comprises the classic endocrine receptors that binds steroid and thyroid hormones as well as vitamin A and D derivatives. The second subgroup are the so called orphan receptors where the ligands have not been identified yet. Members of this subgroup have been identified to bind dietary lipids and metabolites as ligands [23]. Typically, NRs homo- or heterodimerize after ligand binding. The NR dimer travels from the cytoplasm into the nucleus, binds to its specific consensus sequence in the target gene promoters and modulates (activate or repress) gene expression. The recognition sites for NRs comprise typically a pair of 5 to 6 bp long DNA sequences (two half sites) which are often separated by a spacer of 1 to 6 bases of length.

2.4 Molecular biological mechanisms behind the pathogenesis of IBD

The inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC) are characterized by chronic recurrent inflammation of the gastrointestinal tract. Especially young people, age 20 to 40, are affected, showing a strong loss in life quality with work inability and repeated bowel surgeries as well as an putatively elevated risk for the development of colorectal cancer [24; 25]. Curative therapy strategies for IBD do not yet exist. The exact molecular pathogenesis of IBD is not yet fully understood. IBD is thought to be of multifactorial genesis, whereby environmental, microbial, immune and genetic factors seem to play an important role. Current evidence suggests that an initiator (commensal microorganisms within intestinal lumen or their by-products) in association with the disruption of the intestinal epithelium drive a dysregulated immune response in predisposed individuals [26]. This concept is supported by several observations. It could be shown that genetic polymorphisms in genes expressing pro- and anti-inflammatory acting cytoplasmatic receptors that react on bacterial lipopolysaccharides (i.e. nucleotide oligomerisation domain (NOD) 2/caspase recruitment domain (CARD) 15 and NOD1/CARD4) are important susceptibility factors for development of CD [27- 30]. Current therapy approaches in IBD focus on a control of ongoing autoinflammatory processes within the bowel endothelium by using drugs that inhibit the production or the action of cytokines. This includes the single or combined use of steroids, 5-amino salicylic acid (5-ASA), different immunosuppressants (azathioprine, methotrexate) or biologicals (Anti-TNF α -antibodies (-abs)).

The applicant has addressed the pathogenetic complexity of IBD by undertaking different research approaches investigating genetic factors that putatively contribute to the pathophysiology or the treatment outcome with immunosuppressively acting therapeutics in IBD (paper 3 to 5). Here, the impact of a polymorphic expression of two important nuclear receptors involved in immunomodulatory pathways, PPAR γ (Peroxisome proliferator-activated receptor) and GR α (glucocorticoid receptor α) as well as of the cytoplasmically circulating Vitamine D binding protein (DBP), on IBD susceptibility, disease course and therapy success was studied.

3. Discussion of the selected publications

3.1 Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics (Paper 1)

Hitherto, the habilitant was involved in several projects investigating the value of pharmacogenetic biomarkers on drug safety and efficacy *in vivo*. In an important clinical trial we investigated the influence of the often occurring genetic variants *SLCO1B1**1b (rs2306283, c.388A>G) and *5 (rs4149056, c.521T>C) on pravastatin pharmacokinetics. At the time of study performance several *in vitro* studies hinted to the fact that these OATP1B1 polymorphisms were able to modulate the transport efficacy of OATP1B1 *in vitro* [7]. Within the drug family of statins, pravastatin shows

unique disposition kinetics as pravastatin is not transformed by drug metabolizing enzymes and, thus, the extent of hepatic uptake correlates direct inversely with the abundance of the drug in the plasma. In the here presented *in vivo* trial we included 30 healthy individuals, who were either carriers of the alleles *SLCO1B1*1b*, *5 in hetero- or homozygous form or homozygous carriers of the wild type allele. The habilitant and her research group demonstrated that the variant *c.521T>C* is associated with significant lower OATP1B1 uptake transport capacity for pravastatin into the liver, which was associated with significantly higher plasma concentrations of this drug in *SLCO1B1*5* gene variant carriers. In contrast, the carriers of the genetic variant *SLCO1B1*1b* showed lower pravastatin levels compared to wild type carriers. These novel findings were later repeatedly confirmed by other research groups [31; 32]. In a follow up study the habilitant and her research group could later show that the described pharmacokinetic effects were associated with a modulated lipid lowering efficacy of pravastatin. In concordance with the abundance of pravastatin in plasma, lipid lowering effects, as monitored by the measurement of lathosterol and cholesterol plasma levels, were highest in *SLCO1B1*5* variant carriers, followed by wild type and *SLCO1B1*1b* carriers [33]. In a comprehensive case-control study performing a genome wide association analysis (GWAS) involving 300 000 candidate genes and 85 cases and 90 controls it was demonstrated later that the *SLCO1B1* genetic polymorphism *5 dependent changes in the pharmacokinetics of statins are closely related to a significantly higher risk for statin induced myopathies [10]. Based on the repetitively confirmed association between a polymorphic expression of OATP1B1, pravastatin kinetics and its modulated effect on drug efficacy and safety [10; 34; 35] the link between the appearance of *SLCO1B1* genetic variants and a modulated risk for statin induced myopathies is well established today. The risk modulating effect of *SLCO1B1* polymorphisms has, meanwhile, also been confirmed for other members of the statin family, such as e.g. simvastatin [36].

3.2 Regulation of CYP2C19 expression by estrogen receptor alpha. Implications for estrogen dependent inhibition of drug metabolism (Paper 2)

In several *in vitro* studies, the habilitant and her research group were able to shed light on the functional mechanisms on occurring drug-drug interactions, i.e. the putative mechanism by which oral contraceptives are able to inhibit the metabolism of widely used therapeutics, such as proton-pump inhibitors or coumarin derivatives. Beside a polymorphic expression, CYP2C19 enzyme activity is known to be modulated by different drugs, a mechanism, which might be associated with a higher risk of adverse drug effects or with a failure of therapeutic efficacy of in parallel given CYP2C19 substrates [1; 37]. It has been repeatedly demonstrated that the intake of female sex steroids in the form of oral contraceptives (OC) leads to an inhibition of the metabolic efficacy towards CYP2C19 substrates, such as e.g. omeprazole [38; 39]. The inhibitory effect of oral contraceptives is not exclusively limited to CYP2C19 but has also observed with other CYP

enzymes, such as CYP2C9 or CYP2A6 [40; 41]. Despite the fact that, each year, approximately 70 million women worldwide take oral contraceptives, the mechanism by which estrogens, especially the estradiol derivative 17 α -ethinylestradiol affect CYP2C19 activity had not been clarified [42]. Estrogens exert their effects via binding to Estrogen Receptors (ERs). The Estrogen receptors α and β belong to the class of nuclear receptors that act as transcription factors upon dimerization and interaction with specific palindromically featured DNA binding sites (consensus sequence 5'-AGGTCA nnn TGACCT-3') – also called estrogen responsive elements (EREs) - or the respective binding half-sites within the gene promoter targets. While estrogens act as ER agonists, other substances are able to purely (e.g. ICI 182,780) or partially (e.g. tamoxifen or raloxifene) exert estrogen-antagonistic effects by binding to ERs [43; 44].

Based on the hypothesis that an interaction between female sex steroids and CYP2C19 might appear due to Estrogen Receptor-dependent regulatory pathways, the habilitant and her colleagues investigated the ability of estradiol derivatives and the partial estrogen antagonists tamoxifen and raloxifene to modulate CYP2C19 gene expression. The habilitant was able to detect *in silico* an ER binding motif within the CYP2C19 gene promoter. Using important *in vitro* techniques for studying gene regulation, including cell line based co-transfection techniques and luciferase gene reporter assays, TaqMan® analysis, electromobility shift assays and ChIP analysis, the research group could demonstrate that estradiol derivatives have indeed the potential to significantly suppress CYP2C19 expression via binding to ER α - an effect which is reversible by the partial ER antagonists 4-OH-tamoxifen (active metabolite of tamoxifen) or raloxifene. In a follow-up study the habilitant was able to show that the functionally relevant ER binding half site detected within CYP2C19 gene promoter appears to be conserved within other CYP2C gene family members, mediating also ER α dependent effects of female sex steroids on CYP2C9 gene expression [45]. Thus, in paper 2 the habilitant were able to demonstrate that the mechanisms of OC-dependent inhibition of CYP2C19 activity *in vivo* is likely to be mainly exerted by an inhibition of CYP2C19 gene transcription. The detected mechanism has putatively model character for a general principle, how estradiol derivatives might unfold their inhibitory potential on other members of the CYP enzyme family as repeatedly observed in different *in vivo* studies.

3.3 Association of a common vitamin D-binding protein (DBP) polymorphism with inflammatory bowel disease (Paper 3)

Until recently, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃, calcitriol), the active form of vitamin D, was thought to play primarily a role as one of the key regulators in calcium and phosphate metabolism, thereby influencing bone homeostasis. However, the observation that the vitamin D effect mediating protein, vitamin D receptor (VDR) is also expressed in immune cells (e.g. monocytes, antigen presenting cells) led to the hypothesis that vitamin D might have a more universal physiological function, including a regulatory role within the immune system [46; 47]. The effect of

vitamin D is strongly dependent on exposure to ultraviolet light, which initiates photochemical conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D₃) in the skin. In liver and kidney vitamin D₃ is hydroxylated to the main metabolite calcitriol, whose blood concentration is well reflecting the overall vitamin D status of an individual. Binding of vitamin D to VDR induces heterodimerization of VDR with the retinoid X receptor. The protein receptor complex subsequently binds to the vitamin D response elements (VDRE) within its gene promoter targets and modulates gene expression [48]. Interestingly, IBD is more prevalent in regions (e.g. northern Europe and North America) where vitamin D is less efficiently synthesized in the skin due to less exposure to sunlight [49; 50]. Furthermore, there is a strong correlation between the occurrence of IBD with malabsorption symptoms due to small-bowel resection and/ or the use of cholestyramine and vitamin D deficiency [51]. While it has already previously been suggested that genetic variants in the VDR gene are associated with IBD [52], in the work presented here our research group studied for the first time, whether a polymorphic expression of the human Vitamin D-binding protein (DBP) has an impact on IBD development. DBP, also called DBP-macrophage activating factor (DBP-MAF), is encoded by a gene on chromosome 4. It locates in circulating plasma, where it mediates 85% vitamin D metabolite transport to target tissues. Furthermore it is known to have many other important biological functions, including e.g. the mediation of bone resorption by osteoclast differentiation, a direct activation of osteoclasts and macrophages as well as the mediation of neutrophil chemotaxis [53; 54]. There are three common isoforms of the DBP known, namely GC1F (wild type form), GC1S (polymorph at SNP locus rs4588 leading to the amino acid exchange Asp416Glu) and GC2 (polymorph at SNP locus rs7041 leading to the amino acid exchange Thr420Lys). In populations with European ancestry, either for SNPs rs4588 and/or rs7041, the allele with a lower frequency is consistently associated with lower 25(OH)D concentrations [55]. In paper 3 we focussed on the DBP isoform determining [54] common polymorphisms rs7041 and rs4588 and determined their frequency in 636 IBD patients and 248 non-IBD controls. Interestingly, the variant Thr420Lys (isoform GC2) appeared significantly more abundant in the non-IBD control cohort, which would speak for a protective effect against both CD and UC. This observation is surprising, especially with regard to the fact that this variant has been shown to be less effective in vitamin D₃ binding and to be associated with lower vitamin D₃ plasma levels. A logical conclusion might be that the genetic variant Thr420Lys putatively leads to an enhanced release of vitamin D from DBP with subsequent protective effects of still unknown mechanism in the intestinal mucosa.

3.4 Glucocorticoid receptor gene haplotype structure and steroid therapy outcome in IBD patients (Paper 4)

Glucocorticoids (GCs) are widely used as efficient therapeutics to treat chronic inflammatory diseases such as rheumatoid arthritis or inflammatory bowel diseases. The drug group exerts its immune suppressive effects by a potent inhibition of T cell activation and cytokine secretion. This is

reached via a binding of Glucocorticoids to the glucocorticoid receptor (GR) and a subsequent promoter-dependent modulation of gene expression of different target genes, which leads e.g. to a transrepression of AP-1 and NF- κ B signalling pathways [23; 56]. Mechanistically, steroids bind to the cytoplasmically located glucocorticoid receptor, which homodimerizes and translocates into the nucleus. Here, the protein-receptor complex subsequently targets - together with multiple co-regulating proteins - specific glucocorticoid response elements (GREs, 5'-GGTACAnnnTGTCT-3' where n refers to any nucleotide) within the regulatory regions of GR regulated genes and modulates their expression. GCs are often used in the initial treatment of most cases of moderately to severely active UC or CD. However, 20% of patients develop GC resistance within one year of treatment, leading to the need for a surgical intervention as a result of a poor therapy outcome. The mechanisms by which GC resistance develops are, however, not yet fully understood [57; 58]. Three possible mechanisms of how GC resistance develops have been proposed. First, decreased plasma levels of GCs through overexpression of the drug efflux system P-glycoprotein (MDR1). Second, an altered function of GR or, third, excessive synthesis of pro-inflammatory cytokines induced by activation of pro-inflammatory transcription factors may reduce the affinity of GR to its ligands and lead to the development of GC resistance [59].

In paper 4 the habilitant and her research group investigated the impact of occurring genetic polymorphisms within the *NR3C1* gene encoding for Glucocorticoid receptor α on the therapy efficacy of steroids in IBD patients. The research group comprehensively described for the first time all occurring genetic variants and arising haplotypes within the whole exome of *NR3C1*. For this purpose, all exonic regions and flanking intronic regions of *NR3C1* were sequenced in IBD patients, who had formerly responded to a GC treatment () and in GC non-responders () suffering from IBD. The habilitant detected the occurring genetic variants using the blasting algorithm (www.ncbi.gov), bioinformatically predicted the occurring haplotypes of the GR α gene using the software tool Haploview and investigated them in context to therapy outcome with glucocorticoids. The research group could show that GR α appears to be strongly conserved and only rarely polymorphically expressed. Genetic variants within *NR3C1* appeared not to be differentially distributed within GC responders and non-responders, thus, providing evidence that a polymorphic expression of GR α does not serve as a susceptibility marker for a GC associated therapy failure in Crohn's Disease or Ulcerative Colitis.

3.5 The impact of PPAR γ genetic variants on IBD susceptibility and IBD disease course (Paper 5)

Current evidence suggests that PPAR γ , a nuclear receptor and key regulator of genes involved in lipid metabolism and insulin sensitization plays an important role in the regulation of inflammatory processes. By modulating the expression of important transcription factors and kinases involved in inflammatory signalling cascades (e.g. NF- κ B, c-Jun, and c-Fos), PPAR γ is able to inhibit the mucosal production of important proinflammatory cytokines (e.g. interleukin 1 β (IL-1 β) and tumor

necrosis factor- α (TNF α)) and to downregulate the expression of various adhesion molecules [60] [61; 62] By it, PPAR γ is able to efficiently reduce the severity of intestinal inflammation by suppressing excessive immunoinflammatory responses e.g. in the gastrointestinal tract, as demonstrated in mice experiments [63; 64]. Because of its apparent significant impact on the regulation of proinflammatory processes in the colon, we hypothesized that PPAR γ could be a putative susceptibility gene for the development of IBD. In paper 5, the habilitant and her research team comprehensively described for the first time all occurring genetic variants and arising haplotypes in the whole exome of the PPAR γ encoding gene *NR1C3*. This was accomplished by sequencing all exonic and the flanking intronic regions of the most abundantly expressed splice variant PPAR γ 2 in a reasonably large IBD Swiss case and non-IBD control group (140 UC, 144 CD patients and 194 healthy individuals, respectively). The sequencing results were blasted against the reference sequence and the frequencies of the occurring *NR1C3* gene variants were determined in each group. Haplotype and diplotype frequencies were bioinformatically predicted and compared in case and control group using the software tool FAMHAP. Interestingly, the PPAR γ gene appeared to be sparsely polymorphically expressed. Only two genetic variants that were earlier described in the literature appeared to occur in an allelic frequency > 1%. None of the genetic variants or the arising haplo- and diplotypes were associated with a modulated risk for IBD development. Neither were the genetic variants associated with a modulated IBD course, as tested by stratification according to clinical disease activity, fistula state or the occurrence of extraintestinal manifestations. Thus, the performed study proved that a polymorphic expression of PPAR γ does not serve as a susceptibility marker for the development of IBD or disease activity.

4. Value of the presented work and future perspectives

The studies presented here have yielded important insights into the involvement of transcriptional regulatory pathways and genetic variants in drug safety and efficacy and in the pathogenesis of IBD. Specifically, the project results have provided mechanistic explanations of how drug side effects by a polymorphic expression of drug transporters may occur or how drug-drug interactions may develop, giving the possibility to significantly lower the risk of severe side drug side effects based on the gained knowledge. Additionally the overall SNP composition of two important nuclear receptors were extensively studied, which is potentially not only of value with regard to the the investigations made in the context of IBD pathogenesis and treatment but may also be of relevance for other diseases of chronic inflammatory origin.

5. References

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ZUSAMMENFASSUNG DER HABILITATIONSSCHRIFT

Pharmacogenetics of Nuclear Receptors and Drug Transporters in Inflammatory Bowel Disease and hepatic drug metabolism

zur Erreichung der *Venia Legendi*
der Universität Zürich

vorgelegt von
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Zürich, 30. Oktober 2011

Selected Publications Representing the "Kumulative Habilitationsschrift"

The following six publications have been selected as a documentation of my research activities in the field of pharmacogenetics and therapy optimization in IBD:

Mwinyi J, John A, Bauer S, Roots I and Gerloff T (2004) Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics. *Clin Pharmacol Ther* **75**:415-421.
IF (2010)=6.378

Mwinyi J, Cavaco I, Pedersen RS, Persson A, Burkhardt S, Mkrtchian S and Ingelman-Sundberg M (2010) Regulation of CYP2C19 expression by estrogen receptor alpha. Implications for estrogen dependent inhibition of drug metabolism. *Mol Pharmacol* **78**:886-94.
IF (2010)=4.725

Eloranta JJ*, Wenger C*, **Mwinyi J**, Hiller C, Gubler C, Vavricka SR, Fried M, Kullak-Ublick GA, and the Swiss IBD Cohort Study Group (2011) Association of a common vitamin D-binding protein (DBP) polymorphism with inflammatory bowel disease. *Pharmacogenet Genomics*. 2011 Sep;21(9):559-564.
IF (2010)=3.865

Mwinyi J, Wenger C, Eloranta JJ and Kullak-Ublick GA (2010) Glucocorticoid receptor gene haplotype structure and steroid therapy outcome in IBD patients. *World J Gastroenterol* **16**:3888-3896.
IF (2010)=2.092

Mwinyi J*, Grete-Wenger C*, Eloranta JJ, and Kullak-Ublick GA (2011) The impact of PPAR γ genetic variants on IBD susceptibility and IBD disease course. Accepted for publication in the Journal *PPAR Research*
IF (2010)=2.798

Three publications of high scientific value, not discussed in the "Habilitationsschrift"

Baldwin RM*, Ohlsson S*, Pedersen RS*, **Mwinyi J**, Ingelman-Sundberg M, Eliasson E and Bertilsson L (2008) Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. *Br J Clin Pharmacol* **65**:767-774.
(IF (2010)=3.063)

Mwinyi J*, Nekvindova J*, Cavaco I, Hofmann Y, Pedersen RS, Landman E, Mkrtchian S and Ingelman-Sundberg M (2010c) New insights into the regulation of CYP2C9 gene expression: the role of the transcription factor GATA-4. *Drug Metab Dispos* **38**:415-421. IF (2010)=3.716

Mwinyi J*, Cavaco I*, Yurdakok B, Mkrtchian S and Ingelman-Sundberg M (2011) The ligands of estrogen receptor α regulate cytochrome P4502C9 (CYP2C9) expression. *J Pharmacol Exp Ther*. 2011 Jul;338(1):302-9.
IF (2010)=4.017

Background to the presented work

Pharmacogenetics. Many genetic variants, e.g. in genes encoding for cytochrome P450 enzymes or for hepatic transporters have been shown to essentially influence the individual susceptibility for drug side effects. The research field of pharmacogenetics investigates to which extent genetic polymorphisms modulate patients' individual responses to medical treatments.

OATP1B1 and CYP2C19. The hepatically expressed transport protein OATP1B1 is involved in the active uptake of a variety of different drugs from the portal blood into the liver, including e.g. the HMG-CoA reductase inhibitor pravastatin. Especially three non-synonymous SNPs, the genetic variants *SLCO1B1**1*b*, *3 and *5 occur in a high frequency within the *SLCO1B1* gene and have been shown *in vitro* to modulate the OATP1B1 drug transport efficacy. The Cytochrome P450 family member CYP2C19 is as well mainly expressed in the liver and metabolizes about 5% of currently used therapeutics, including proton pump inhibitors. The coding gene *CYP2C19* is highly polymorphically expressed with high impact on drug safety and efficacy. While CYP2C19 slow metabolizers (carriers of the SNP *2 or *3) have been shown to be at higher risk for thrombotic events under treatment with the anticoagulative acting prodrug clopidogrel, ultrafast metabolizers (genetic variant *17) appear to be at lower risk for thrombotic events under clopidogrel.

The transcription factor family of Nuclear Receptors: Nuclear receptors are involved in the regulation of a wide range of metabolic pathways via ligand-dependent activation and induction of transcription changes of different genes. NRs typically recognize a pair of 5 to 6 bp long DNA sequences, which are separated by a spacer of 1 to 6 bases of length. Based on the type of ligand specificity NRs are divided into three subfamilies, including classic endocrine receptors that bind steroid and thyroid hormones and vitamine A and D derivatives, orphan receptors, for which the ligands have not been identified yet and receptors, which bind dietary lipids and their metabolites.

Molecular biological mechanisms behind the pathogenesis of IBD: The inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory disease of the gastrointestinal tract, which are associated with a strong loss in life quality, work inability, repeated bowel surgeries and a putatively elevated risk for the development of colorectal cancer. Curative therapy strategies for IBD do not yet exist. Not yet exactly elucidated, the pathogenesis of IBD is thought to be of multifactorial genesis, whereby environmental, microbial, immune and genetic factors seem to play a key role. Current therapy approaches in IBD focus on a control of ongoing autoinflammatory processes within the bowel endothelium by using drugs that inhibit the production or the action of cytokines, which includes the use of e.g. steroids, 5-amino salicylic acid (5-ASA), immunosuppressants (azathioprine) or biologicals (Anti-TNF α -antibodies (-abs)).

Discussion of the selected publications

Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics (Paper 1): In this study it was investigated to which extent the genetic variants *SLCO1B1**1*b*

(rs2306283, c.388A>G) and *5 (rs4149056, c.521T>C) of the gene encoding for the liver uptake transporter OATP1B1 influence the pharmacokinetics of pravastatin *in vivo*. The trial included 30 healthy white individuals, either carriers of the alleles *SLCO1B1**1*b*, *5 or homozygous carriers of the wild type allele, who were treated with a single dose of 40mg pravastatin. The polymorphism c.521T>C appeared to be associated with a significant lower OATP1B1 uptake transport capacity for pravastatin into the liver, as demonstrated by significantly higher pravastatin plasma concentrations (AUC_{0-6h}) in *SLCO1B1**5 carriers compared to wild type carriers. In contrast, carriers of *SLCO1B1**1*b* showed lower pravastatin levels compared to wild type carriers, as demonstrated in lower pravastatin concentrations in 24h-urine. This was the first study, which proved a significant impact of OATP1B1 variants on an HMG-CoA-inhibitor *in vivo*.

Regulation of CYP2C19 expression by estrogen receptor alpha. Implications for estrogen dependent inhibition of drug metabolism (Paper 2): Beside a polymorphic expression, the activity of the hepatic drug metabolizing enzyme CYP2C19 is known to be modulated by different drugs, leading to important drug-drug interactions. The intake of oral contraceptives (OCs) inhibits the metabolic efficacy of CYP2C19 *in vivo*. To elucidate the mechanism behind this inhibitory effect, the habilitant investigated the ability of estradiol derivatives and partial estrogen antagonists to modulate *CYP2C19* gene expression via an Estrogen Receptor (ER) dependent regulatory pathway. Initially, several putative ER binding motifs within the *CYP2C19* gene promoter were detected *in silico*. Using important *in vitro* techniques, such as cell line based co-transfection techniques and luciferase gene reporter assays, electromobility shift assays and ChIP and TaqMan® analysis, it could be shown that estradiol derivatives are able to significantly inhibit *CYP2C19* gene expression via a functionally active ER α – binding half site within the first 200bp of the *CYP2C19* promoter, an effect, which was reversible by the partial antagonist 4-OH-tamoxifen. The detected mechanism has putatively model character for a general principle, how estradiol derivatives might unfold their inhibitory potential on other members of the CYP enzyme family.

Association of a common vitamin D-binding protein (DBP) polymorphism with inflammatory bowel disease (Paper 3): Vitamin D is a key regulator in calcium and phosphate homeostasis. Its effect is mediated by the Vitamin D receptor, which binds vitamin D and subsequently modulates gene expression of different target genes. Vitamine D binding protein (DBP) is localized in human plasma, where it mediates 85% vitamin D transport to its target tissues. Furthermore, DBP is able to directly activate macrophages and to stimulate chemotaxis of neutrophil granulocytes. Interestingly, there is a strong correlation between the prevalence of IBD and the occurrence of vitamine D deficiency. In Paper 3 the habilitant investigated to which extent a polymorphic expression of the human Vitamin D-binding protein (DBP) has an impact on IBD development, investigating the prevalence of the two DBP isoform determining common genetic variants rs7041 and rs4588 in 636 IBD patients and 248 non-IBD controls. The polymorphism rs4588, which has earlier been shown to be less effective in vitamin D₃ binding, was significantly less frequent in the

IBD patient group Further studies have to elucidate in which way the enhanced rs4588 triggered release of vitamin D from DBP exerts the apparent protective effect observed in this study.

Glucocorticoid receptor gene haplotype structure and steroid therapy outcome in IBD patients (Paper 4): Glucocorticoids (GCs) are efficient drugs in the treatment of inflammatory bowel diseases, exerting a potent inhibition of T cell activation and cytokine secretion via a modulation of glucocorticoid receptor α (GR α) dependent signalling pathways. A major problem in IBD treatment is that 20% of patients develop GC resistance within one year of treatment. In paper 4 the habilitant studied the impact of genetic polymorphisms within the Glucocorticoid receptor α gene *NR3C1* on the therapy outcome in IBD patients treated with steroids. All occurring genetic variants and arising haplotypes within *NR3C1* were determined by whole exome sequencing and subsequent haplotype analysis in IBD patients, who had formerly responded to a GC treatment (R) and in GC non-responders (NR). Interestingly, GR α appeared to be only rarely polymorphically expressed. Genetic variants within *NR3C1* were not differentially distributed between GC responders and non-responders, thus, providing evidence that GR α polymorphisms do not serve as a susceptibility marker for a GC associated therapy failure in IBD.

The impact of PPAR γ genetic variants on IBD susceptibility and IBD disease course (Paper 5): PPAR γ belongs to the nuclear receptor family and is key regulator of inflammatory processes by modulating the expression of transcription factors and kinases involved in the inhibition of proinflammatory signalling cascades. To investigate whether a polymorphic expression of PPAR γ could serve as susceptibility marker for IBD the habilitant comprehensively determined the genetic variants and arising haplotypes within the whole exome of the PPAR γ encoding gene *NR1C3* by sequencing all exonic and the flanking intronic regions of the most abundantly expressed splice variant PPAR γ 2 and predicting bioinformatically the occurring haplotypes in a reasonably large IBD case and non-IBD control group (294 IBD patients and 194 healthy individuals). Interestingly, the PPAR γ gene appeared to be sparsely polymorphically expressed. Only two genetic variants occurred in an allelic frequency > 1%. Neither the detected polymorphisms nor the predicted haplotypes were associated with a modulated risk for IBD development or disease course, thus proving, that PPAR γ polymorphisms do not modulate the individual risk for IBD.

Value of the presented work

The studies presented here have yielded important insights into the involvement of transcriptional regulatory pathways and genetic variants in drug safety and efficacy and in the pathogenesis of IBD. Specifically, the project results have provided important mechanistic explanations of how drug side effects by a polymorphic expression of drug transporters may occur or how drug-drug interactions may develop.